Autochthonous and Dormant *Cryptococcus gattii* Infections in Europe

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Until recently, *Cryptococcus gattii* infections occurred mainly in tropical and subtropical climate zones. However, during the past decade, *C. gattii* infections in humans and animals in Europe have increased. To determine whether the infections in Europe were acquired from an autochthonous source or associated with travel, we used multilocus sequence typing to compare 100 isolates from Europe (57 from 40 human patients, 22 from the environment, and 21 from animals) with 191 isolates from around the world. Of the 57 human patient isolates, 47 (83%) were obtained since 1995. Among the 40 patients, 24 (60%) probably acquired the *C. gattii* infection outside Europe; the remaining 16 (40%) probably acquired the infection within Europe. Human patient isolates from Mediterranean Europe clustered into a distinct genotype with animal and environmental isolates. These results indicate that reactivation of dormant *C. gattii* infections can occur many years after the infectious agent was acquired elsewhere.

During the past decade, the basidiomycetous yeast *Cryptococcus gattii* has stepped out of the shadows of its sibling *C. neoformans*. The latter species mainly infects immunocompromised persons, and *C. gattii* mainly infects apparently immunocompetent persons. *C. neoformans* is found globally, and *C. gattii* has been mostly limited to tropical and subtropical areas in Central Africa, northern Australia, and Central and South America (*1*). However, this distribution pattern changed after an unprecedented outbreak of *C. gattii* emerged in the temperate climate of British Columbia, Canada, and expanded to the Pacific Northwest region of Canada and the United States (*1*,*2*). Epidemiologic studies have shown that *C. gattii* occurs in areas other than tropical or subtropical zones, such as in Mediterranean Europe, northern Europe, and western Australia (*3*–*5*).

For the purpose of studying the epidemiology of *C. gattii*, a broad variety of molecular biological techniques have been developed, including PCR fingerprinting, restriction fragment length polymorphism analysis of the *PLB1* and *URA5* loci, amplified fragment length polymorphism (AFLP) fingerprint analysis, and several multilocus sequence typing (MLST) approaches (*6*–*9*). These laboratory investigations have shown that *C. gattii* can be divided into 5 distinct genotypes: AFLP4/VGI, AFLP5/VGIII, AFLP6/VGII, AFLP7/VGIV, and AFLP10/VGIV (*8*,*9*). Serotype B strains occur in genotypes AFLP4/VGI, AFLP6/VGII, and AFLP10/VGIV; serotype C strains are restricted to genotypes AFLP5/VGIII and AFLP7/VGIV (*8*).

Recently, a consensus MLST scheme was proposed for epidemiologic investigations of *C. gattii* and *C. neoformans*, specifically, the nuclear loci *CAP59*, *GPD1*, IGS1, *LAC1*, *PLB1*, *SOD1*, and *URA5* (*9*). So far, this consensus MLST scheme has been used to study the population structure of *C. neoformans* strains from Thailand and *C. gattii* strains from Australia (*3*,*10*).

We investigated the occurrence of *C. gattii* in Europe, focusing on whether this pathogen is emerging and, if so, how to explain this emergence pattern. Furthermore, we

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explored whether the infections originated from Europe or were introduced from other continents. To achieve these goals, members of the European Confederation of Medical Mycology were asked to send recently obtained human patient isolates of the species for detailed AFLP and MLST analyses. Thus, the genetic diversity of the yeast was used to trace its geographic origin to identify where the infections were acquired. AFLP genotyping results were combined with published *C. gattii* MLST results from Byrnes et al. (*11*) and Fraser et al. (*12*), which were extended to match the *Cryptococcus* consensus MLST scheme (*9*). Our study produced the following 5 conclusions: all hitherto known genotypes of *C. gattii* are emerging in Europe; genotype AFLP4/VGI isolates predominate; a *C. gattii* cluster, which is endemic to Mediterranean Europe and genetically distinct from the other populations, exists; several human infections are caused by travel-related acquisition of *C. gattii* outside Europe; and autochthonous cases occur in Europe.

Materials and Methods

Strains and Media

We compared a collection of 107 isolates collected from Europe with 194 isolates collected globally (Table 1; online Technical Appendix Table 1, wwwnc.cdc.gov/EID/pdfs/12-0068-Techapp.pdf). All isolates were checked for purity and cultivated on malt extract agar medium (Oxoid, Basingstoke, UK). Cultures were incubated for 2 days at 30°C. A working collection was made by growing *C. gattii* strains on malt extract agar slants for 2 days at 30°C, after which the strains were stored at 4°C. Strains were stored long term at −80°C by using the Microbank system (Pro-Lab Diagnostics, Richmond Hill, Ontario, Canada).

Amplification and Sequencing of MLST Loci

Genomic DNA extraction, AFLP genotyping, and mating-type determination by amplification of either the *STE12***a** or *STE12*α locus were performed as described (*8*). The 7 nuclear consensus MLST loci (*CAP59*, *GPD1*, IGS1, *LAC1*, *PLB1*, *SOD1*, and *URA5*) were amplified by using the preferred primer combinations (*9*). To compare the current set of *C. gattii* strains with those from a published *C. gattii* population biology study, we included the 3 nuclear loci that are not part of the consensus MLST scheme (*CAP10*, *MPD1*, and *TEF1*α) (*12*). We also included isolates from a global study by Byrnes et al. (*11*) and Fraser et al. (*12*) and subjected them to amplification and sequencing of the *CAP59*, *SOD1*, and *URA5* loci.

Amplifications were conducted in a 25-μL PCR mixture containing 37.5 mmol/L $MgCl_2$ (Bioline, London, UK), 1× PCR buffer (Bioline), 1.9 mmol/L dNTPs (Bioline), 0.5 U Taq DNA polymerase (Bioline), 5 pmol of both primers (Biolegio, Nijmegen, the Netherlands) (online Technical Appendix Table 2), and ≈100 ng of genomic DNA. PCRs were conducted with an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30s, annealing for 30s (see Technical Appendix Table 2 for optimal annealing temperatures), extension at 72°C for 1 min, followed by 72°C for 5 min and a final dwell at 21°C.

Sequencing reactions were conducted with the BigDye version 3.1 chemistry kit (Applied Biosystems, Foster City, CA, USA) as described (*13*). For all amplification products except *CAP59*, the initial amplification primers were used for sequencing reactions. For *CAP59*, the newly designed forward primer CAP59L-Fwd and the original reverse primer JOHE15438 (*12*) were used.

Sequence Alignment and Phylogenetic and Recombination Analyses

Consensus sequences were assembled and checked for ambiguities by using SeqMan version 8.0.2 (DNASTAR, Madison, WI, USA). Sequence alignments were generated with MEGA version 5 (*14*) by using the standard settings and manual correction. The genome sequence databases of reference strains H99 (culture collection no. CBS8710; *C. neoformans* variety *grubii*; AFLP1/VNI; Broad Institute [www.broadinstitute.org/annotation/genome/cryptococcus_neoformans/]) and JEC21 (culture collection no. CBS10513; *C. neoformans* variety *neoformans*; AFLP2/VNIV; Stanford Genome Technology Center [www-sequence.stanford.edu/group/C.neoformans/]) were used to extract the corresponding sequences for all 10 investigated nuclear loci to serve as an outgroup. The best fitting nucleotide substitution model was determined by using MrModeltest version 2 (*15*) and was conducted for the complete *C. gattii* 10-loci MLST, for the accepted consensus MLST scheme (*CAP59*, *GPD1*, IGS1, *LAC1*, *PLB1*, *SOD1*, and *URA5*) (*9*), and a previously launched *C. gattii* MLST scheme (*CAP10*, *GPD1*, IGS1, *LAC1*, *MPD1*, *PLB1*, and *TEF1*) (*12*). As a result, the HKY G+I model (Hasegawa-Kishino-Yano plus gamma distributed with invariant sites) was the best model to use for analyzing the phylogeny of the *C. gattii* isolates for all 3 datasets. The evolutionary history was inferred by using the maximum-likelihood method in MEGA version 5 (*14*). A bootstrap consensus tree was inferred from 1,000 replicates to show the relevant lineages obtained in this analysis.

We calculated the haplotype diversity (H_R), equal to the Simpson diversity index (*D*), by using the Microsoft Excel (Microsoft, Redmond, WA, USA) add-in called Haplotype Analysis (*16*). For this purpose, sequences were collapsed into sequence type numbers (online Technical Appendix Table 1).

Results

AFLP Genotypes and Geographic Distribution

The 301 *C. gattii* isolates collected from Europe and other areas around the world could be divided into the following genotypes: 146 AFLP4/VGI (50.2%; 72 from Europe), 22 AFLP5/VGIII (7.6%; 1 from Europe), 108 AFLP6/VGII (37.1%; 23 from Europe), 13 AFLP7/VGIV (4.5%; 2 from Europe), and 2 AFLP10/VGIV (0.7%; both from Europe). From 10 isolates (7 AFLP8 [5 from Europe] and 3 AFLP9 [2 from Europe]), genotypes represented interspecies *C. neoformans* × *C. gattii* hybrids. These 10 isolates were excluded from further analysis because amplified fragments of hybrid isolates will result in mixtures of different alleles. The 57 human patient isolates from Europe were obtained from 40 patients (online Technical Appendix Table 1). The genotypic diversity of the remaining 291 *C. gattii* isolates (100 from Europe, 191 from other areas) with a haploid genotype is shown in the Table.

Phylogeographic Origin

Sequence type diversity was calculated for each of the MLST loci, all 10 loci, and the combined datasets according to Fraser et al. (*12*) and Meyer et al. (*9*) (online Technical Appendix Table 3). The *CAP10* locus showed the lowest overall diversity (n_{ST} = 19; D_{ST} = 0.765) and the IGS1 locus showed the highest diversity (n_{ST} = 52; D_{ST} = 0.930). The 10-loci MLST dataset showed the highest diversity (n_{ST} = 150; D_{ST} = 0.975), followed by the MLST scheme of Meyer et al. (D_{ST} = 0.971) (*9*) and Fraser et al. (D_{ST} of 0.959) (*12*). The latter MLST scheme differentiated more sequence types than the consensus MLST scheme (n_{ST} = 136 vs. 127).

Maximum-likelihood analysis of the MLST data showed that the *C. gattii* isolates clustered in 5 monophyletic clusters, which were highly supported and agreed with the AFLP genotypes (Figure; online Technical Appendix Figure). When each genotypic cluster was separately analyzed, support values of the branches were low, <75

Table. Distribution of *Cryptococcus gattii* strains*

Source of isolation	Genotype: AFLP4/VGI	AFLP5/VGIII	AFLP6/VGII	AFLP7/VGIV	AFLP10/VGIV	Total†	Total (%)
All *C. gattii* isolates	146	22	108	13	2	0	291 (100)
Human	84	16	68	12	2	0	182 (62.5)‡
Environment	37	5	17	0	0	0	59 (20.3)‡
Animal	24	0	23	1	0	0	48 (16.5)‡
Unknown	1	1	0	0	0	0	2 (0.7)‡
Africa							
Human	18	0	3	8	0	29	36 (12.7)§
Environment	6	0	0	0	0	6	
Animal	0	0	0	1	0	1	
Asia (clinical)	19	0	5	2	0	26	26 (8.9)§
Australia							
Human	2	1	8	0	0	11	18 (6.2)§
Environment	5	0	1	0	0	6	
Animal	0	0	1	0	0	1	
Europe¶							
Human	29#	1**	23††	2‡‡	2§§	57	100 (34.4)§
Environment	22	0	0	0	0	22	
Animal	21	0	0	0	0	21	
North America¶							
Human	3	8	19	0	0	30	69 (23.7)§
Environment	4	3	10	0	0	17	
Animal	2	0	20	0	0	22	
South America¶							
Human	12	3	10	0	0	25	36 (12.4)§
Environment	0	2	6	0	0	8	
Animal	1	0	2	0	0	3	
Unknown							
Human	1	3	0	0	0	4	6 (2.1)§
Unknown	1	1	0	0	0	2	

*The number of *C. gattii* isolates is provided for each geographic area and further subdivided per genotype and source of isolation. AFLP, amplified fragment length polymorphism; human, human clinical patient.
†Total number of isolates per geographic area.
‡The percentage of isolates is given for the source of isolation subset.
§The percentage of isolates is given as a percentage of all isolates.
¶Ten interspecies hybrid *C. gattii* × *C. neoformans* isolates were excluded from the set of isolates from Europe (n = 7), North America (n = 1), and South America (n = 2).
#Twelve patients with an autochthonous acquired infection (16 isolates) and 11 patients with an infection acquired outside Europe (13 isolates).
**Infection acquired outside Europe.
††Four patients with an autochthonous infection (10 isolates) and 10 patients with an infection acquired outside Europe (13 isolates).
‡‡Two phenotypically different isolates from an emigrant from Zambia.
§§Two phenotypically different isolates from an emigrant from Mexico.

(online Technical Appendix Figure). For genotype AFLP4/VGI isolates, bootstrap support values for nearly all branches were low; for the second largest group formed by AFLP6/VGII isolates, branches were better supported.

Phylogenetic analysis demonstrated that several human patient genotype AFLP4/VGI isolates from Europe had an autochthonous origin because they formed a separate cluster with environmental and animal isolates from the same area. A set of isolates from human patients on the Iberian Peninsula (CCA232, CCA242L, CCA242T, CCA311, CCA312, CL6148) were found to be genetically indistinguishable from isolates obtained from animals or the environment in the same area (indicated in online Technical Appendix Figure as the European Mediterranean cluster). The human patient isolates from Europe with genotype AFLP4/VGI, which were probably acquired within Europe because patients did not have histories of travel outside Europe, are IP2005/215 and IP2006/958 (France), RKI85/888 (Germany), 5UM and 75UM (Italy), RKI97/482 (Portugal), and CBS2502 (the Netherlands). Some of these human patient isolates were closely related (e.g., IP2005/215 and RKI97/482) or even genetically indistinguishable (e.g., CBS2502 and RKI85/888).

A large proportion of the human patient and environmental isolates of *C. gattii* AFLP4/VGI from Italy and Spain formed a novel autochthonous Mediterranean MLST cluster that was genetically homogeneous, irrespective of their origin or mating type (Figure, online Technical Appendix Figure). *C. gattii* AFLP4/VGI was involved with numerous small outbreaks among goats in Spain, and the isolates were genetically indistinguishable from recently obtained human patient, animal, and environmental isolates from different provinces in Spain as well as from AFLP4/VGI mating-type **a** isolates from Italy (online Technical Appendix Figure).

Several *C. gattii* genotype AFLP6/VGII infections were found to have originated in Europe (e.g., the IP1998/1037–1 and –2, IP2003/125, and CCA242 isolates), and all fell within a cluster that could not be linked to an environmental source. The same holds true for the human patient isolates from Greece (AV54S, –W, and IUM01–4731), which came from the same patient who had no history of travel outside Greece.

In the phylogenetic analysis, isolates from human patients in Europe were also observed next to those originating from other geographic areas. The most striking example was a set of 4 human patient isolates from citizens of Denmark, the Netherlands, Germany, and Switzerland, in whom cryptococcosis developed after they had visited Vancouver Island, British Columbia, Canada. The isolates obtained from these tourists (CBS10485, RKI06/496, RKI01/774, and CBS10866, respectively) had MLST profiles identical to that of *C. gattii* AFLP6A/VGIIa, the genotype that caused the Vancouver Island outbreak (Figure 1; online Technical Appendix Figure) (*17–19*). The outbreak-related sets of AFLP6A/VGIIa and AFLP6C/VGIIc isolates from the Vancouver Island and Pacific Northwest outbreaks, respectively, are within outbreak-specific clusters (Figure, online Technical Appendix Figure). A similar finding was observed for a set of 5 human patient *C. gattii* AFLP6B/VGIIb isolates (IP1996/1120–1 and –2, IP1999/901–1 and –2, and IP2000/87) in France,

Figure. Maximum-likelihood phylogenetic analysis based on 10-loci multilocus sequence type data of *Cryptococcus gattii* isolates (condensed). Phylogenetic relatedness of 150 STs representing the 291 *C. gattii* isolates, calculated by using the maximum-likelihood algorithm and rooted by using the 2 *C. neoformans* reference strains CBS8710 (genotype AFLP1/VNI) and CBS10513 (genotype AFLP2/VNIV). Closely related sequence types were collapsed into 1 branch shown by multiple sequence type numbers. **Boldface** indicates sequence types that are within a shaded area belong to a specified *C. gattii* cluster; B, M, PNW, and VIO represent clusters from Brazil; Mediterranean Europe; the US Pacific Northwest outbreak; and the Vancouver Island, British Columbia, Canada, outbreak, respectively. AFLP, amplified fragment length polymorphism; ST, sequence type. Scale bar indicates number of substitutions per site. See online Technical Appendix Figure (wwwnc.cdc.gov/EID/pdfs/12-0068-Techapp.pdf) for a detailed phylogenetic analysis.

which had been obtained from patients who had emigrated from Africa to France and which were genetically indistinguishable from an isolate (IP2001/935–1) from a resident of Senegal (online Technical Appendix Figure). This set of 5 isolates from France and 1 from Senegal were closely related to isolates from the Vancouver Island and Pacific Northwest outbreaks; however, it seems unlikely that the patient from Senegal had traveled to these outbreak areas, and none of the patients in France reported having traveled to Canada or the United States.

A set of *C. gattii* AFLP4/VGI mating-type α isolates from Portugal (IP1997/18) and Belgium (IHEM19725B and –S) was found to be indistinguishable from human patient isolates from the Democratic Republic of the Congo and Rwanda (B3939 and CBS6289, which were both mating-type **a**, and IHEM10602S, IHEM10769S, and IHEM10769W, which were mating-type α) (Figure; online Technical Appendix Figure). Both phenotypically different isolates of IHEM19725 were obtained from an HIV-infected patient from Rwanda who had emigrated to Belgium. The CBS1622 isolate from Europe, obtained from a patient with the oldest documented case of a *C. gattii* infection (*20*), was found to be genetically indistinguishable from a set of isolates from North America.

The single *C. gattii* AFLP5/VGIII isolate from a 24-year-old immunocompetent patient from Germany (isolate RKI97/310) (*21*) clustered with human patient and environmental isolates from Mexico (online Technical Appendix Figure). The 2 *C. gattii* AFLP7/VGIV isolates CBS7952D and CBS7952S, obtained from an HIV-infected patient in Sweden, were genetically indistinguishable from each other and had a unique MLST profile that clustered with human patient isolates from Africa. According to the patient's history, years before the onset of cryptococcal infection, she had emigrated from Zambia to Sweden (online Technical Appendix Figure). Another example of a reactivated dormant *C. gattii* infection is that of the human patient isolate from Italy, IUM92–6682 (AFLP4/VGI), obtained from an immunocompetent immigrant from Brazil, which had an identical MLST profile to 6 human patient mating-type α isolates (IHEM14934, IHEM14956, IHEM14965, IHEM14968, IHEM14976, and IHEM14984) from Brazil (Figure, online Technical Appendix Figure).

Infection was acquired outside Europe for 24 of these patients (31 isolates) and within Europe for 16 patients (26 isolates) (Table; online Technical Appendix Figure). Among these 57 human patient isolates, most (47 [82.5%]) were obtained since 1995, the remaining 10 (17.5%) were isolated during 1895–1994. One of these isolates originated from 1985, another 2 isolates (CBS1622 and CBS2502), from 1895 and 1957, were retrospectively found to represent *C. gattii* isolates from Europe (*20*,*22*).

Discussion

In Europe, *C. gattii* has been reported as a rare cause of apparently autochthonous cryptococcal infections (*1*). The earliest documented case that turned out to be caused by *C. gattii* in Europe was made by Curtis in 1896 (*20*). Until the 1980s, cryptococcosis was rarely observed in Europe and *C. gattii* infections were especially rare; only 2 cases have retrospectively been found (*20*,*22*). In the 1980s, infections were reported for 2 immunocompetent citizens of Germany, who had never traveled abroad (*23*,*24*). Subsequent case reports described *C. gattii* infections that were imported or acquired in Europe (*25*,*26*). Since 1995, the number of reports of *C. gattii* infections increased and describe *C. gattii* infections in humans and animals from Greece (*27*), Italy (*28*,*29*), and Spain (*5*,*30*–*33*). In the current study, 8 (20%) cases were observed until 1995, and 32 (80%) cases in humans have been observed since 1995. We excluded cases for which an isolate was not available for confirmation. This exclusion is especially relevant in that we re-identified a reported *C. gattii* infection as actually being caused by *C. neoformans* (*34*). These data suggest that during the past 2 decades, *C. gattii* has been emerging in Europe.

In the current study, 16 (40%) of the human patient isolates from Europe were found to have an autochthonous origin in Europe that either could be linked to environmental isolates from the Mediterranean area or that came from patients who had never traveled outside their resident country. A total of 24 (60%) isolates could be linked to *C. gattii*–endemic regions in Brazil, the United States, Africa, and the Vancouver Island outbreak region. These observations demonstrate that *C. gattii* infections can be imported subclinically and can cause infections after being dormant for many years.

Nearly all isolates from Mediterranean Europe belonged to genotype AFLP4/VGI, and MLST analysis showed that these isolates form a separate cluster within this genotype (Figure; online Technical Appendix Figure) (*5*). *C. gattii* genotype AFLP4/VGI has also recently been reported from the environment in the Netherlands, but these isolates were not similar to any of the AFLP4/VGI isolates from Mediterranean Europe or to isolate CBS2502, which was isolated in 1957 from a pregnant citizen of the Netherlands, who had never traveled abroad but who died of cryptococcosis (*4*). Isolate CBS2502 is genotypically identical to isolate RKI85/888, which was isolated from a previously healthy citizen of Germany, who also had never traveled outside Germany. These 2 cases strongly suggest that different genotypes of *C. gattii* AFLP4/VGI occur in the environment of northwestern Europe because isolates from patients were genetically different from the recently reported isolates from the environment of the Netherlands (this study; *4*).

In conclusion, *C. gattii* is emerging in Europe, and the isolates from Europe can be divided into 5 genotypic clusters. Most *C. gattii* infections in Europe are probably autochthonous, and several infections are proven to have been acquired outside the European continent, e.g., during visits to *C. gattii*–endemic regions, such as the Vancouver Island outbreak area or before migration to Europe from *C. gattii*–endemic regions in Africa and South America. Reactivation of dormant *C. gattii* infections and of infections acquired outside Europe after immune suppression occurs more often than previously assumed. This finding might suggest that *C. gattii* infections caused by certain genotypes are associated with altered immune status of the human host. Thus, *C. gattii* is probably a more opportunistic pathogen, as has recently been hypothesized, than a strictly primary pathogen (*18*,*35*). *C. gattii* isolates with genotypes AFLP5/VGIII and AFLP7/VGIV were rarely found in Europe and were all acquired outside the European continent. However, these genotypes have frequently been isolated from HIV-infected persons and other immunocompromised patients in Africa and the American continents (*1*,*7*,*36*). A connection seems to exist between these *C. gattii* genotypes and the host's immune status. Further epidemiologic and immunologic research is needed to unravel this apparent correlation.

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Autochthonous and Dormant *Cryptococcus gattii* Infections in Europe

Technical Appendix

Figure (see last page). Maximum-likelihood phylogenetic analysis based on 10-loci multilocus sequence typing data of *Cryptococcus gattii* isolates (detailed). Phylogenetic relatedness of 291 *C. gattii* isolates calculated by using the maximum-likelihood algorithm with 1,000 bootstraps and rooted by using the 2 *C. neoformans* reference strains CBS8710 (genotype AFLP1/VNI) and CBS10513 (genotype AFLP2/VNIV). The 5 *C. gattii* AFLP genotypes AFLP4/VGI, AFLP5/VGIII, AFLP6/VGII, AFLP7/VGIV, and AFLP10/VGIV are highly supported with bootstrap values of ≥75, as indicated next to the branches. Isolate numbers that are within a shaded area belong to a specified *C.* gattii cluster. Amplified fragment length polymorphism (AFLP) genotype clusters are indicated within a colored box as follows: red, AFLP4/VGI; blue, AFLP10/VGIV; green, AFLP5/VGIII; purple, AFLP7/VGIV; orange, AFLP6/VGII. Colors of the isolate number and locality of isolation refer to their source as follows: red, clinical; blue, animal; green, environmental; black, unknown source. Clinical isolates from Europe that have an autochthonous origin are indicated with a red asterisk, those isolates that were probably acquired outside the European continent are indicated with a blue hash mark. Scale bar indicates number of substitutions per site.

Table 1. Background information of *Cryptococcus gattii* isolates and GenBank accession numbers for the 10-loci multilocus sequence typing data*

Population	Source	Source/Remark	Publication	PMID number
North America	Veterinary	Canine, isolated at 17/01/2007	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Veterinary	Feline, isolated at 10/02/2006	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Veterinary	Canine, isolated at 10/02/2006	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Veterinary	Canine, isolated at 11/03/2006	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Veterinary	Feline, isolated at 13/04/2006	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Veterinary	Feline, isolated at 13/04/2006	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Veterinary	Canine, isolated at 04/04/2006	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Veterinary	Feline, isolated at 11/07/2006	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
South America	Veterinary	Cheetah (Zoological Park of La Habana, Cuba; Cheetah was imported from South Africa)	Illnait-Zaragozi et al. Mycoses. 2011;54:e889-e892	21668523
North America	Veterinary	Feline, isolated at 29/06/2007	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Veterinary	Canine, isolated at 12/06/2007	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Veterinary	Canine, isolated at 30/06/2007	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Veterinary	Canine, isolated at 29/06/2007	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Veterinary	Feline, isolated at 15/03/2008	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Veterinary	Feline, isolated at 10/06/2008	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Europe	Environmental	*Eucalyptus camaldulensis* leaf (isolated 11/06/1996)	Montagna et al. J Mycol Méd. 1997;7:93-96	n/a
Europe	Environmental	*Eucalyptus camaldulensis* flower (isolated 11/06/1996)	Montagna et al. J Mycol Méd. 1997;7:93-96	n/a
Europe	Environmental	*Eucalyptus camaldulensis* bark (isolated 11/06/1996)	Montagna et al. J Mycol Méd. 1997;7:93-96	n/a
North America	Clinical	Clinical	Ganz et al. J Clin Invest. 1985;76:1427-1435	2997278
Europe	Veterinary	Ostrich (*Struthio camelus*) feather (isolated 11/06/1996)	Montagna et al. J Mycol Méd. 1997;7:93-96	n/a
South America	Environmental	Nest of *Polybia occidentalis* (communal wasp; 1989)	Gezuele et al. Rev Iberoam Micol. 1993;10:5-6.	n/a
Unknown	Clinical	Clinical	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Veterinary	Goat lung, Pescueza outbreak	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Veterinary	Goat lung, Pescueza outbreak	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Veterinary	Goat lung, Serradilla outbreak	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Veterinary	Goat lung, Serradilla outbreak	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Veterinary	Camel (*Camelus bactrianus*) hair (1996)	Montagna et al. J Mycol Méd. 1997;7:93-96	n/a
Europe	Veterinary	Goat liver, Casas de Millan outbreak	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Veterinary	Goat CSF, Casas de Millan outbreak	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Veterinary	Goat lung, Madronera outbreak	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Veterinary	Goat lung, Madronera outbreak	Colom et al. Med Mycol. 2012;50:67-73	21521012

Population	Source	Source/Remark	Publication	PMID number
Europe	Veterinary	Brain tissue of a goat	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Veterinary	Goat lung, Vera outbreak	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Veterinary	Goat lung, Vera outbreak	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Veterinary	Goat lung, Vera outbreak	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Clinical	CSF from human (1995)	Montagna et al. J Mycol Méd. 1997;7:93-96	n/a
Europe	Veterinary	Goat lung, Vera outbreak	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Veterinary	Goose egg (1996)	Montagna et al. J Mycol Méd. 1997;7:93-96	n/a
Europe	Environmental	Small lake with a black swan (*Cygnus atratus*) (1997)	Montagna et al. J Mycol Méd. 1997;7:93-96	n/a
Europe	Environmental	Water in nandu (*Rhea americana*) and emu (*Dromaius novaehollandiae*) cage (1997)	Montagna et al. J Mycol Méd. 1997;7:93-96	n/a
Europe	Environmental	Water in a emu (*Dromaius novaehollandiae*) cage (1997)	Montagna et al. J Mycol Méd. 1997;7:93-96	n/a
Europe	Clinical	CSF from a 37y old male with cirrhosis, had frequently contact with birds/pigeons (2009)	Iatta et al. Mycopathologia. 2012;in press	22057831
Europe	Environmental	Small lake near peacock (*Pavo cristatus*) cage (1997)	Montagna et al. J Mycol Méd. 1997;7:93-96	n/a
Europe	Environmental	Soil from peacock (*Pavo cristatus*) cage (1997)	Montagna et al. J Mycol Méd. 1997;7:93-96	n/a
Europe	Veterinary	Squirrel (*Sciurus* spp.) faeces	Montagna et al. J Mycol Méd. 1997;7:93-96	n/a
Europe	Environmental	Small lake with a black swan (*Cygnus atratus*) (1997)	Montagna et al. J Mycol Méd. 1997;7:93-96	n/a
Europe	Environmental	Water from parrots (Psittaciformes) drinking place (1997)	Montagna et al. J Mycol Méd. 1997;7:93-96	n/a
Asia	Clinical		Taylor et al. J Clin Microbiol. 2002;40:3098-3099	12149391
North America	Clinical	Dead wild Dall's porpoise lumph node autopsy	Kidd et al. Proc Natl Acad Sci USA. 2004;101:17258-17263	15572442
North America	Clinical	Lung tissue from an immunocompetent male	Kidd et al. Proc Natl Acad Sci USA. 2004;101:17258-17263	15572442
North America	Clinical	Dead wild Dall's porpoise mediastinal lymph node	Kidd et al. Proc Natl Acad Sci USA. 2004;101:17258-17263	15572442
North America	Clinical	Bronchial wash from an immunocompetent male	Kidd et al. Proc Natl Acad Sci USA. 2004;101:17258-17263	15572442
North America	Clinical	Bronchial wash from an immunocompetent female	Kidd et al. Proc Natl Acad Sci USA. 2004;101:17258-17263	15572442
North America	Clinical	Bronchial alveolar lavage from an immunocompetent male	Kidd et al. Proc Natl Acad Sci USA. 2004;101:17258-17263	15572442
North America	Clinical	Bronchial alveolar lavage from an immunocompetent female	Kidd et al. Proc Natl Acad Sci USA. 2004;101:17258-17263	15572442
North America	Clinical	Clinical	Kidd et al. Proc Natl Acad Sci USA. 2004;101:17258-17263	15572442
North America	Clinical	CSF from an immunocompetent female	Kidd et al. Proc Natl Acad Sci USA. 2004;101:17258-17263	15572442
North America	Clinical	CSF from an immunocompetent female	Kidd et al. Proc Natl Acad Sci USA. 2004;101:17258-17263	15572442
Africa	Clinical	CSF from a HIV-positive patient	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Clinical	Human, geographic grid USGS45122-C5D8 (2005)	Byrnes et al. J Infect Dis. 2009;199:1081-1086	19220140
North America	Clinical	Human (2006)	Byrnes et al. J Infect Dis. 2009;199:1081-1086	19220140
North America	Clinical	Unknown	Evans. Proc Soc Exp Biol Med. 1949;71:644-646	18148185
North America	Clinical	CSF from a 45y-old immunocompetent female, traveled to California (1968) and Mexico (1979 and 1980) (1984)	Bottone et al. J Clin Microbiol. 1986;23:186-188	3517042
Europe	Clinical	CSF of HIV-negative human (isolate AV54S, AV54W and IUM01-4731 are subcultures) (2001)	Bovers et al. Fungal Genet Biol. 2008;45:400-421	18261945
Europe	Clinical	CSF of HIV-negative human (isolate AV54S, AV54W and IUM01-4731 are subcultures) (2001)	Bovers et al. Fungal Genet Biol. 2008;45:400-421	18261945

Population	Source	Source/Remark	Publication	PMID number
Europe	Clinical	Skin of HIV negative human (isolate AV55 and IUM00-5363 are subcultures) (2000)	Bovers et al. Fungal Genet Biol. 2008;45:400-421	18261945
Africa	Clinical	CSF from a HIV-positive patient	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Africa	Clinical	CSF from a HIV-positive patient	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Africa	Clinical	CSF from a HIV-positive patient	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Africa	Clinical	CSF of a male patient	Gatti and Eeckels. Ann Soc Belges Med Trop Parasitol Mycol. 1970;50:689-693	5519205
Asia	Clinical	CSF of 30-yr-old immunocompetent nurse with chronic meningitis	Padhye et al. J Med Vet Mycol. 1993;31:165-168	8509953
Asia	Clinical	Swelling below the knee, 17-year-old male (unknown HIV-status)	Padhye et al. J Med Vet Mycol. 1993;31:165-168	8509953
Africa	Clinical	CSF from a HIV-positive patient	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Africa	Environmental	Tree hollow of an *Eucalyptus* sp. tree in the centre of Cape Town	This study	n/a
Africa	Environmental	Tree hollow of an *Eucalyptus* sp. tree in the centre of Cape Town	This study	n/a
Africa	Environmental	Tree hollow of an *Eucalyptus* sp. tree in the centre of Cape Town	This study	n/a
Africa	Environmental	Tree hollow of an *Eucalyptus* sp. tree in the centre of Cape Town	This study	n/a
Africa	Environmental	Tree hollow of an *Eucalyptus* sp. tree in the centre of Cape Town	This study	n/a
Africa	Environmental	Tree hollow of an *Eucalyptus* sp. tree in the centre of Cape Town	This study	n/a
Europe	Clinical	CSF of 51y HIV negative male who traveled to Vancouver Island (2005)	Lindberg et al. Emerg Infect Dis. 2007;13:179-179	17370544
Africa	Clinical	CSF from patient with unknown HIV-status	This study	n/a
Asia	Clinical	CSF from HIV- 39y female (2001)	Lui et al. QJM. 2006;99:143-151	16504989
Asia	Clinical	CSF from HIV- 40y male (2003)	Lui et al. QJM. 2006;99:143-151	16504989
Asia	Clinical	CSF from HIV- 34y male (1998)	Lui et al. QJM. 2006;99:143-151	16504989
Asia	Clinical	CSF from HIV- 34y male (1998)	Lui et al. QJM. 2006;99:143-151	16504989
Asia	Clinical	CSF from HIV- 34y male (1998)	Lui et al. QJM. 2006;99:143-151	16504989
Asia	Clinical	CSF from HIV- 34y male (1998)	Lui et al. QJM. 2006;99:143-151	16504989
Europe	Clinical	BAL of 26y female with SLE (isolate CBS10608 and IUM98-1969 are subcultures) (1998)	Velegraki et al. Med Mycol. 2001;39:419-422	12054052
Europe	Clinical	CSF of 31y caucasian male (1996)	Velegraki et al. Med Mycol. 2001;39:419-422	12054052
Europe	Clinical	CSF from HIV- 31y male (2003)	This study	n/a
Europe	Clinical	CSF from a 42-year-old female Dutch resident with SLE who visited Vancouver Island (2007)	Hagen et al. Med Mycol. 2010;48:528-531	19824880
North America	Clinical	CSF from a healthy 45-year-old female resident of Alberta who frequently visited Vancouver Island (2006)	Levy et al. Can J Infect Dis Med Microbiol. 2007;18:197-199	18923724
North America	Clinical	CSF from a healthy 45-year-old female resident of Alberta who frequently visited Vancouver Island (2006)	Levy et al. Can J Infect Dis Med Microbiol. 2007;18:197-199	18923724
Australia	Clinical	Human	Katsu et al. FEMS Yeast Res. 2004;4:377-388	14734018
Europe	Clinical	CSF of immunocompetent child (1989)	Guinea et al. Med Mycol. 2010;48:942-948	20297948
Asia	Clinical	Clinical	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Asia	Clinical	Clinical	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Europe	Clinical	Tumour like structure, human (1895)	Curtis. C r de la Soc de Biol. 1895;2:715-718	n/a
South America	Veterinary	Sick goat	Diaz et al. Syst Appl Microbiol. 2000;4:535-545	11249024
North America	Veterinary	Cow with mastitis (1952)	Boekhout et al. Int J Syst Bacteriol. 1997;47:432-442	9103633
Europe	Clinical	Isolated from a 27y old 34w pregnant women, fatal case of cryptococcosis (1957)	Janssens and Beetstra. Ned Tijdschr Geneeskd. 1957;101:824-826	13451717

Population	Source	Source/Remark	Publication	PMID number
Unknown	Unknown	Unknown	Boekhout et al. Int J Syst Bacteriol. 1997;47:432-442	9103633
Unknown	Unknown	Unknown	Boekhout et al. Int J Syst Bacteriol. 1997;47:432-442	9103633
Africa	Clinical	CSF of man	Gatti and Eeckels. Ann Soc Belges Med Trop Parasitol Mycol. 1970;50:689-693	5519205
Africa	Clinical	Human	Boekhout et al. Microbiology. 2001;147:891-907	11283285
North America	Clinical	CSF of patient with cryptococcal meningitis	Boekhout et al. Microbiology. 2001;147:891-907	11283285
North America	Clinical	Sputum, immunocompetent human	Boekhout et al. Microbiology. 2001;147:891-907	11283285
North America	Clinical	Human	Boekhout et al. Microbiology. 2001;147:891-907	11283285
North America	Clinical	CSF from a human	Boekhout et al. Microbiology. 2001;147:891-907	11283285
North America	Clinical	CSF of a male patient	Boekhout et al. Microbiology. 2001;147:891-907	11283285
North America	Clinical	Clinical	Diaz et al. Syst Appl Microbiol. 2000;4:535-545	11249024
North America	Clinical	CSF of a male patient	Boekhout et al. Microbiology. 2001;147:891-907	11283285
Asia	Clinical	CSF of a male patient (1978)	Diaz et al. Syst Appl Microbiol. 2000;4:535-545	11249024
Asia	Clinical	CSF of 42y male (1980)	Boekhout et al. Microbiology. 2001;147:891-907	11283285
Australia	Environmental	Bark debris of *Eucalyptus camaldulensis* (river red gum tree)	Boekhout et al. Microbiology. 2001;147:891-907	11283285
Asia	Clinical	CSF of patient with chronic myeloid leukaemia (1984)	Boekhout et al. Microbiology. 2001;147:891-907	11283285
Asia	Clinical	CSF of patient with chronic meningitis (1987)	Boekhout et al. Microbiology. 2001;147:891-907	11283285
Asia	Clinical	CSF of patient with chronic meningitis (1987)	Boekhout et al. Microbiology. 2001;147:891-907	11283285
Australia	Environmental	Seedling of olive, under canopy of *Eucalyptus camaldulensis* (1989)	Boekhout et al. Microbiology. 2001;147:891-907	11283285
Australia	Environmental	Amosphere in hollow *Eucalyptus camaldulensis*	Boekhout et al. Microbiology. 2001;147:891-907	11283285
Australia	Environmental	Bark of *Eucalyptus camaldulensis*	Boekhout et al. Microbiology. 2001;147:891-907	11283285
North America	Environmental	*Eucalyptus camaldulensis* bark debris (1990)	Boekhout et al. Microbiology. 2001;147:891-907	11283285
Unknown	Clinical	Filamentous mutant of CBS7812 (=clinical *C. gattii*)	This study	n/a
Europe	Clinical	CSF from HIV-positive 41y female who emigrated from Zambia (1995)	This study	n/a
Europe	Clinical	CSF from HIV-positive 41y female who emigrated from Zambia (1995)	This study	n/a
South America	Environmental	Nest of *Polybia occidentalis* (communal wasp; 1989)	Gezuele et al. Rev Iberoam Micol. 1993;10:5-6.	n/a
Asia	Clinical	CSF from 28-year-old man	This study	n/a
South America	Environmental	Litter of *Prunus dulcis* (Almond tree)	Boekhout et al. Microbiology. 2001;147:891-907	11283285
South America	Environmental	Litter of *Prunus dulcis* (Almond tree)	Boekhout et al. Microbiology. 2001;147:891-907	11283285
Unknown	Clinical	Infected skin from man	Boekhout et al. Microbiology. 2001;147:891-907	11283285
North America	Clinical	Meningo-encephalitic lesion in man (1924)	Boekhout et al. Microbiology. 2001;147:891-907	11283285
Europe	Clinical	Drainage of brain-abcess of HIV-negative 60-year-old Spanish farmer with diabetes mellitus type 2 (2003)	Colom et al. J Clin Microbiol. 2005;43:3548-3550	16000503
Europe	Clinical	Clinical (isolate CCA242A, CCA242G, CCA242O and CCA242X are subcultures) (2005)	This study	n/a
Europe	Clinical	Clinical (isolate CCA242A, CCA242G, CCA242O and CCA242X are subcultures) (2005)	This study	n/a
Europe	Clinical	Clinical (isolate CCA242L, CCA242N and CCA242T are subcultures) (2005)	This study	n/a
Europe	Clinical	Clinical (isolate CCA242L, CCA242N and CCA242T are subcultures) (2005)	This study	n/a
Europe	Clinical	Clinical (isolate CCA242A, CCA242G, CCA242O and CCA242X are subcultures) (2005)	This study	n/a
Europe	Clinical	Clinical (isolate CCA242L, CCA242N and CCA242T are subcultures) (2005)	This study	n/a
Europe	Clinical	Clinical (isolate CCA242A, CCA242G, CCA242O and CCA242X are subcultures) (2005)	This study	n/a
Europe	Veterinary	Ferret	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Veterinary	Ferret nasal swab (asymptomic carrier)	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Veterinary	Ferret nasal swab (asymptomic carrier)	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Veterinary	Ferret nasal swab (asymptomic carrier)	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Clinical	Human nasal swab (asymptomic carrier) (2010)	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Clinical	Human nasal swab (asymptomic carrier) (2010)	Colom et al. Med Mycol. 2012;50:67-73	21521012

Population	Source	Source/Remark	Publication	PMID number
Europe	Environmental	Bark swab	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Environmental	Bark swab	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Environmental	Bark swab	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Environmental	Detritus swab	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Environmental	Detritus swab	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Environmental	Detritus swab	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Environmental	Bark swab	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Environmental	Bark swab	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Environmental	Detritus swab	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Environmental	Detritus swab	Colom et al. Med Mycol. 2012;50:67-73	21521012
Europe	Clinical	CSF of immunocompromised 66-year-old Spanish male with SLE (2008)	Solla et al. Enferm Infecc Microbiol Clin. 2008;26:395-396	18588823
Australia	Clinical	CSF from human	Bovers et al. Fungal Genet Biol. 2008;45:400-421	18261945
South America	Environmental	Native tree	Baltazar and Ribeiro. Rev Soc Bras Med Trop. 2008;41:449-453	19009184
Asia	Clinical	CSF from a HIV- 33y old Malaysian male	Koh et al. Med Mycol. 2002;40:221-223	12058737
Asia	Clinical	CSF from a HIV- 69y old Singaporean Male	Koh et al. Med Mycol. 2002;40:221-223	12058737
Australia	Environmental		Halliday et al. J Clin Microbiol. 1999;37:2920-2926	10449476
North America	Clinical	Human	Byrnes et al. J Infect Dis. 2009;199:1081-1086	19220140
North America	Veterinary	Veterinary	Byrnes et al. J Infect Dis. 2009;199:1081-1086	19220140
North America	Veterinary	Veterinary	Byrnes et al. J Infect Dis. 2009;199:1081-1086	19220140
North America	Clinical	Human	Byrnes et al. J Infect Dis. 2009;199:1081-1086	19220140
North America	Environmental	Douglas Fir tree #131	Kidd et al. Proc Natl Acad Sci USA. 2004;101:17258-17263	15572442
North America	Environmental	Alder tree #152	Kidd et al. Proc Natl Acad Sci USA. 2004;101:17258-17263	15572442
South America	Clinical	Urine of HIV positive 53y male (1994)	Katsu et al. FEMS Yeast Res. 2004;4:377-388	14734018
South America	Clinical	CSF of an HIV negative human	Barreto de Oliveira et al. J Clin Microbiol. 2004;42:1356-1359	15004118
South America	Environmental	Tree hollow	Barreto de Oliveira et al. J Clin Microbiol. 2004;42:1356-1359	15004118
South America	Environmental	*Eucalyptus* sp. tree	Barreto de Oliveira et al. J Clin Microbiol. 2004;42:1356-1359	15004118
South America	Environmental	*Eucalyptus* sp. tree	Barreto de Oliveira et al. J Clin Microbiol. 2004;42:1356-1359	15004118
South America	Clinical	CSF of an HIV negative human	Barreto de Oliveira et al. J Clin Microbiol. 2004;42:1356-1359	15004118
South America	Clinical	Clinical	Uno et al. Nihon Ishinkin Gakkai Zasshi. 2001;42:127-132	11479533
North America	Clinical	CSF from human (1986)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Africa	Clinical	Human CSF (1986)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Africa	Clinical	CSF of AIDS patient (1990)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Africa	Clinical	CSF of AIDS patient (1990)	Hagen et al. Antimicrobial Agents Chemother.	29755729

Population	Source	Source/Remark	Publication	PMID number
			2010;54:5139-5145	
Africa	Clinical	Blood of AIDS patient (1990)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Africa	Clinical	Blood of AIDS patient (1990)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Africa	Clinical	CSF of AIDS patient (1991)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Africa	Clinical	CSF of AIDS patient (1991)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Africa	Clinical	CSF, human (1969)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Africa	Clinical	CSF, human (1966)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Africa	Clinical	CSF, human (1957)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Africa	Clinical	CSF, human (1953)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Africa	Clinical	CSF, human (1951)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
South America	Clinical	Human (1987)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
South America	Clinical	Human (1987)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Europe	Clinical	HIV- patient, immigrant from Mexico (isolate IHEM14941S and IHEM14941W are subcultures) (1987)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Europe	Clinical	HIV- patient, immigrant from Mexico (isolate IHEM14941S and IHEM14941W are subcultures) (1987)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
South America	Clinical	Non-AIDS-patient (1987)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
South America	Clinical	Human (1988)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
South America	Clinical	Human (1988)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
South America	Clinical	AIDS patient (1988)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
South America	Clinical	Human (1988)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
South America	Clinical	Human (1988)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
South America	Clinical	Human (1988)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
South America	Clinical	AIDS patient (1989)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Europe	Clinical	CSF from an Angolese patient (isolate IHEM16633B and IHEM16633S are subcultures) (2000)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Europe	Clinical	CSF from an Angolese patient (isolate IHEM16633B and IHEM16633S are subcultures) (2000)	Hagen et al. Antimicrobial Agents Chemother.	29755729

Population	Source	Source/Remark	Publication	PMID number
			2010;54:5139-5145	
Europe	Clinical	CSF from a HIV-positive Rwandese patient (isolate IHEM19725B and IHEM19725S are subcultures) (2003)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Europe	Clinical	CSF from a HIV-positive Rwandese patient (isolate IHEM19725B and IHEM19725S are subcultures) (2003)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Asia	Clinical	Human (1984)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Unknown	Clinical	Clinical	Cogliati et al. Mycoses. 2012;In press	21815945
Europe	Clinical	Clinical (1993)	Fraser et al. Nature. 2005;437:1360-1364	16222245
South America	Clinical	CSF of immunocompetent 28y-old male (1989)	Fraser et al. Nature. 2005;437:1360-1364	16222245
South America	Clinical	CSF from immunocompetent 11y-old female (1986)	Fraser et al. Nature. 2005;437:1360-1364	16222245
Europe	Clinical	Clinical (isolate IP1996/1120-1 and IP1996/1120-2 are from the same patient) (1996)	Fraser et al. Nature. 2005;437:1360-1364	16222245
Europe	Clinical	Clinical (isolate IP1996/1120-1 and IP1996/1120-2 are from the same patient) (1996)	Fraser et al. Nature. 2005;437:1360-1364	16222245
South America	Clinical	CSF from immunocompetent 36y-old male (1997)	Fraser et al. Nature. 2005;437:1360-1364	16222245
Europe	Environmental	Environmental	Fraser et al. Nature. 2005;437:1360-1364	16222245
Europe	Environmental	Environmental	Fraser et al. Nature. 2005;437:1360-1364	16222245
South America	Clinical	BAL from immunocompetent 36y-old male (1997)	Fraser et al. Nature. 2005;437:1360-1364	16222245
South America	Clinical	CSF of 39y-old male (1997)	Fraser et al. Nature. 2005;437:1360-1364	16222245
South America	Clinical	CSF of 17y-old female (1997)	Fraser et al. Nature. 2005;437:1360-1364	16222245
Europe	Clinical	Clinical (isolate IP1998/1037-1 and IP1998/1037-2 are from the same patient) (1998)	Fraser et al. Nature. 2005;437:1360-1364	16222245
Europe	Clinical	Clinical (isolate IP1998/1037-1 and IP1998/1037-2 are from the same patient) (1998)	Fraser et al. Nature. 2005;437:1360-1364	16222245
South America	Clinical	Clinical	Fraser et al. Nature. 2005;437:1360-1364	16222245
Europe	Clinical	BAL of 27y old HIV2-positive female (see isolate IP99/901-2) (1999)	Fraser et al. Nature. 2005;437:1360-1364	16222245
Europe	Clinical	Lung biopsy of HIV2-positive 27y-old female (see IP99/901-1) (1999)	Fraser et al. Nature. 2005;437:1360-1364	16222245
Europe	Clinical	Clinical (2000)	Fraser et al. Nature. 2005;437:1360-1364	16222245
Africa	Clinical	Clinical	Fraser et al. Nature. 2005;437:1360-1364	16222245
Europe	Clinical	CSF of immunocompetent 31y-old male (2003)	Fraser et al. Nature. 2005;437:1360-1364	16222245
Europe	Clinical	CSF of 51y-old male with AIDS (2003)	Fraser et al. Nature. 2005;437:1360-1364	16222245
South America	Clinical	Lung of immunocompetent 64y-old male (2004)	Fraser et al. Nature. 2005;437:1360-1364	16222245
South America	Clinical	Lung of immunocompetent 64y-old female (2004)	Fraser et al. Nature. 2005;437:1360-1364	16222245
Europe	Clinical	Lung of immunocompetent 43y-old female (2005)	Fraser et al. Nature. 2005;437:1360-1364	16222245
Europe	Clinical	Lung of immunocompetent 52y-old female (2006)	This study	n/a
Europe	Clinical	CSF from male (2006)	This study	n/a
Europe	Clinical	Skin of HIV negative human (isolate AV55 and IUM00-5363 are subcultures) (2000)	Bovers et al. Fungal Genet Biol. 2008;45:400-421	18261945
Europe	Clinical	CSF of HIV-negative human (isolate AV54S, AV54W and IUM01-4731 are subcultures) (2001)	Bovers et al. Fungal Genet Biol. 2008;45:400-421	18261945
Asia	Clinical	CSF of HIV negative human	Cogliati et al. Mycoses. 2012;In press	21815945
Europe	Clinical	CSF of HIV negative Brazilian patient (resident in Italy) (1992)	Cogliati et al. Mycoses. 2012;In press	21815945
Asia	Clinical	CSF of HIV negative human	Cogliati et al. Mycoses. 2012;In press	21815945
Asia	Clinical	CSF of HIV negative human	Cogliati et al. Mycoses. 2012;In press	21815945
Europe	Clinical	BAL of 26y female with SLE (isolate CBS10608 and IUM98-1969 are subcultures) (1998)	Velegraki et al. Med Mycol. 2001;39:419-422	12054052
North America	Veterinary	Feline, geographic grid NTS092G/01 (2005)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Environmental	Water (2006)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Environmental	Water (2006)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729

Population	Source	Source/Remark	Publication	PMID number
North America	Veterinary	Feline (2006)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Veterinary	Canine (2006)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Veterinary	Feline (2008)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Environmental	Air, geographic grid NTS092G/01 (2004)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
South America	Veterinary	Parrot liver (2000)	Raso et al. Med Mycol. 2004;42:355-362	15473361
Africa	Clinical		Bovers et al. Fungal Genet Biol. 2008;45:400-421	18261945
North America	Clinical	Human (2005)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
North America	Clinical	Human (2005)	Hagen et al. Antimicrobial Agents Chemother. 2010;54:5139-5145	29755729
Australia	Clinical		Fraser et al. Nature. 2005;437:1360-1364	16222245
Australia	Clinical	Human	Fraser et al. Nature. 2005;437:1360-1364	16222245
North America	Veterinary	Lung tissue of 19y-old adult deceased male wild living Atlantic bottlenose dolphin (*Torsiops truncatus*)	Miller et al. J Clin Microbiol. 2002;40:721-724	11826007
Europe	Clinical	CSF from a 47y old HIV-positive male (1994)	Hagen et al. J Clin Microbiol. 2012;submitted	n/a
Australia	Clinical		Fraser et al. Nature. 2005;437:1360-1364	16222245
Australia	Clinical	Human	Fraser et al. Nature. 2005;437:1360-1364	16222245
Australia	Environmental	*Eucalyptus camaldulensis* (1999)	Fraser et al. Nature. 2005;437:1360-1364	16222245
North America	Environmental	Hollow from Douglas Fir tree #113	Kidd et al. Proc Natl Acad Sci USA. 2004;101:17258-17263	15572442
North America	Environmental	Composite swab 2m W of Alder tree #152	Kidd et al. Proc Natl Acad Sci USA. 2004;101:17258-17263	15572442
North America	Environmental	Retest of Douglas Fir #126 (hollow 2: 1/2)	Kidd et al. Proc Natl Acad Sci USA. 2004;101:17258-17263	15572442
North America	Environmental	Retest of Douglas Fir #126 (hollow 2: 1/2)	Kidd et al. Proc Natl Acad Sci USA. 2004;101:17258-17263	15572442
Australia	Clinical	Human	Fraser et al. Nature. 2005;437:1360-1364	16222245
Australia	Clinical	Human	Fraser et al. Nature. 2005;437:1360-1364	16222245
Australia	Clinical	Human	Fraser et al. Nature. 2005;437:1360-1364	16222245
Europe	Clinical	German male who visited Vancouver Island (2001)	This study	n/a
Europe	Clinical	Clinical (2001)	This study	n/a
Europe	Clinical	Suisse female who visited Vancouver Island (2006)	Georgi et al. Infection. 2009;37:370-373	19390780
Africa	Clinical		Lemmer et al. Med Mycol. 2004;42:135-147	15124867
Africa	Clinical		Lemmer et al. Med Mycol. 2004;42:135-147	15124867
Europe	Clinical	45y-old immunocompetent German resident, lung cryptococcoma (asymptomatic), patient never left Germany (1985)	Schnaberg et al. Internist (Berl). 1988;29:510-515	3049426
Africa	Clinical		This study	n/a
South America	Clinical	34y old immunocompetent female from Brazil who was hospitalized in Switzerland	This study	n/a
Europe	Clinical	24y-old immunocompetent German resident who developed multifocal encephalomyelitis (1997)	Grosse et al. J Neurol Neurosurg Psychiatry. 2001;70:113-116	11118259
Europe	Clinical	Clinical (1997)	Lemmer et al. Med Mycol. 2004;42:135-147	15124867
Europe	Clinical	Clinical (1998)	This study	n/a

Population	Source	Source/Remark	Publication	PMID number
Africa	Clinical		Lemmer et al. Med Mycol. 2004;42:135-147	15124867
Africa	Clinical		Lemmer et al. Med Mycol. 2004;42:135-147	15124867
Africa	Clinical		Lemmer et al. Med Mycol. 2004;42:135-147	15124867
Asia	Clinical	Second isolate of *C. neoformans* variety *shanghaiensis*	Liao et al. Chin Med J. 1983;96:287-290	6413143
Asia	Clinical	Second isolate of *C. neoformans* variety *shanghaiensis*	Liao et al. Chin Med J. 1983;96:287-290	6413143
South America	Clinical	CSF of HIV-negative human	This study	n/a
Australia	Veterinary	Koala	Fraser et al. Nature. 2005;437:1360-1364	16222245
North America	Environmental	Tree hollow	Meyer et al. Med Mycol. 2009;47:561-570	19462334
North America	Environmental	*Eucalyptus citriodora*	Boekhout et al. Microbiology. 2001;147:891-907	11283285
Australia	Clinical	Lung of HIV negative human (1991)	Meyer et al. Med Mycol. 2009;47:561-570	19462334
Australia	Clinical	CSF of HIV negative human (1993)	Meyer et al. Med Mycol. 2009;47:561-570	19462334
Australia	Clinical	Woody debris of *Eucalyptus tericornis* (1993)	Meyer et al. Med Mycol. 2009;47:561-570	19462334
North America	Environmental	Woody debris of *Eucalyptus tericornis*	Boekhout et al. Microbiology. 2001;147:891-907	11283285
North America	Environmental	Woody debris of *Eucalyptus tericornis*	Boekhout et al. Microbiology. 2001;147:891-907	11283285
North America	Environmental	*Eucalyptus citriodora*	Boekhout et al. Microbiology. 2001;147:891-907	11283285
North America	Environmental	Debris of *Eucalyptus* from car park of zoo	Boekhout et al. Microbiology. 2001;147:891-907	11283285
North America	Environmental	Debris of *Eucalyptus* from car park of zoo	Boekhout et al. Microbiology. 2001;147:891-907	11283285
Africa	Veterinary	Cheetah (1994)	Meyer et al. Med Mycol. 2009;47:561-570	19462334

*Alternative isolate numbers, background information regarding the source and origin of the isolate, as well as the reference to the literature are provided. For each isolate the GenBank accession numbers are provided for each of the ten MLST loci, as well as the Sequence Types (STs) for each locus and for the combination of the ten loci. The sequence data for the loci *CAP59*, *GPD1*, IGS1, *LAC1*, *PLB1*, *SOD1* and *URA5* has been included in the recently launched MLST database (http://mlst.mycologylab.org/).

Table 2. Primers used for multilocus sequence typing

	Primer sequences for amplification and sequencing			
Locus	Forward primer sequence	Reversed primer sequence	Optimal T_M	Reference
CAP10	CAP10L-Fwd 5′-GTCGTTTTCGCCGATCCTC-3′	CAP10L-Rvd 5′-GCCGTAAGACGTGCCCA-3′	60°C	This study
*CAP59**	CAP59L-Fwd 5′-GTGAACAAGCTGCGGC-3′	CAP59L-Rvd 5′-GGATTCAGTGTGGTGGAAGA-3′	58°C	This study; *1**
GPD1	GPD1L-Fwd 5′-GGTTGTCAAGGTTGGAATCAACGG-3′	GPD1L-Rvd 5′-GGAGCGGAAATGACGACCTTCTT-3′	61°C	This study
IGS1	IGSF 5′-ATCCTTTGCAGACGACTTGA-3′	IGSR 5′-GTGATCAGTGCATTGCATGA-3′	61°C	*2*
LAC1	LAC1F 5′-AACATGTTCCCTGGGCCTGTG-3′	LAC1R 5′-ATGAGAATTGAATCGCCTTGT-3′	50°C	*2*
MPD1	MPD1L-Fwd 5′-CCCAGACTGCCGCTGT-3′	MPD1L-Rvd 5′-GTGCCGCTAGGCTTCAAGTA-3′	51°C	This study
PLB1	PLB1F	PLB1R	56°C	*2*

Locus	Primer sequences for amplification and sequencing: Forward primer sequence	Reversed primer sequence	Optimal T_M	Reference
	5′-CTTCAGGCGGAGAGAGGTTT-3′	5′-GATTTGGCGTTGGTTTCAGT-3′		
SOD1	SOD1CGF	SOD1CGR	52°C	*2*
	5′-GATCCTCACGCCATTACG-3′	5′-GAATGATGCGCTTAGTTGGA-3′		
TEF1	TEF1L-Fwd	TEF1L-Rvd	62°C	This study
	5′-CTCGGACGGCGAATCGACCAAGAGG-3′	5′-GACGGTCAGACCCGAGAGCACGC-3′		
URA5	URA5F	URA5R	63°C	*2*
	5′-ATGTCCTCCCAAGCCCTCGAC-3′	5′-TTAAGACCTCTGAACACCGTACTC-3′		

*Sequences and optimal annealing temperatures of primer combinations used for amplification and sequencing of each of the ten nuclear loci are provided. The same primers were used to amplify and sequence the obtained amplicons, an exception was made for sequencing of the *CAP59* amplification product, the reversed primer JOHE15438 (5′-CTCTACGTCGAGCAAGTCAAG-3′) was used instead of CAP59L-Rvd due to the presence of the mono-nucleotide-region in front of the primer site.

Table 3. Sequence type diversity for each of the investigated loci and AFLP genotype clusters

	CAP10		*CAP59*		*GPD1*		IGS1		*LAC1*		*MPD1*		*PLB1*		*SOD1*		*TEF1*		*URA5*		MLST Fraser		MLST Meyer		All MLST loci	
Genotype	n_{ST}	D_{ST}	n_{ST}	D_{ST}	n_{ST}	D_{ST}	n_{ST}	D_{ST}	n_{ST}	D_{ST}	n_{ST}	D_{ST}	n_{ST}	D_{ST}	n_{ST}	D_{ST}	n_{ST}	D_{ST}	n_{ST}	D_{ST}	n_{ST}	D_{ST}	n_{ST}	D_{ST}	n_{ST}	D_{ST}
AFLP4 (146)	7	0.499	14	0.609	10	0.504	22	0.820	10	0.540	15	0.667	7	0.514	25	0.776	20	0.506	9	0.627	69	0.908	54	0.926	76	0.943
AFLP5 (22)	3	0.567	3	0.567	5	0.805	6	0.840	8	0.848	4	0.732	7	0.749	4	0.403	8	0.823	7	0.779	21	0.996	16	0.974	17	0.978
AFLP6 (108)	6	0.239	13	0.754	12	0.784	19	0.832	9	0.490	2	0.019	14	0.679	22*	0.809*	4	0.464	11	0.632	34	0.872	45	0.924	44	0.924
AFLP7 (13)	4	0.654	9	0.910	5	0.821	4	0.679	3	0.692	1	0.000	4	0.615	8	0.859	6	0.795	2	0.154	11	0.974	11	0.974	12	0.987
All isolates (291)	19	0.765	40	0.863	32	0.844	52	0.930	31	0.813	25	0.776	30	0.829	59	0.913	34	0.790	30	0.853	136	0.959	127	0.971	150	0.975

*The number of strains, between brackets, for each of the AFLP genotypes showing the overall distribution. Genotype AFLP10/VGIV has been omitted as a separate group from this analysis due to the low number of strains (n = (*2*) that were identical to each other for all ten loci. The number of sequence types (nST) and the diversity (DST) is provided for each locus as well as for the combined set of loci as previously used by Fraser et al. (*1*) and Meyer et al. (*1*) and all loci combined (current study). The study by Fraser et al. (*1*) included the loci CAP10, GPD1, IGS1, LAC1, MPD1, PLB1 and TEF1. The consensus MLST scheme by Meyer et al. (*2*) included the loci CAP59, GPD1, IGS1, LAC1, PLB1, SOD1, and URA5. The asterisk highlights the absence of four strains in the analysis of the SOD1 locus for which no amplicon could be obtained (Technical Appendix Table 1).

References

1. Fraser JA, Giles SS, Wenink EC, Geunes-Boyer SG, Wright JR, Diezmann S, et al. Same-sex mating and the origin of the Vancouver Island *Cryptococcus gattii* outbreak. Nature. 2005;437:1360–4. PubMed http://dx.doi.org/10.1038/nature04220

2. Meyer W, Aanensen DM, Boekhout T, Cogliati M, Diaz MR, Esposto MC, et al. Consensus multi-locus sequence typing scheme for *Cryptococcus neoformans* and *Cryptococcus gattii*. Med Mycol. 2009;47:561–70. PubMed http://dx.doi.org/10.1080/13693780902953886


AFLP4/VGI
European Mediterranean cluster
Brazilian cluster
AFLP10/VGIV
AFLP5/VGIII
AFLP7/VGV
AFLP6/VGII
Pacific Northwest outbreak cluster
Vancouver Island outbreak cluster
CBS10913 (AFLP3/VNIV)
CBS6710 (AFLP1/VNI)
0.02